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THE FORMATION OF THE NOTOCHORD IN THE AMPHIBIA.

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A study of the mode of development of the notochord in the common toad, *Bufo lentiginosus*, and of the frog, *Rana palustris*, has brought to light certain points that have a bearing on the formation of the same structure in related groups. A vast amount of work has already been done along this line, yet a wide difference of opinion exists among embryologists regarding the origin of the notochord in the Amphibia. It is hoped that the results recorded in the present paper may help to clear up this question.

The material used was fixed in corrosive-acetic (5° glacial acetic acid), and the sections were stained on the slide with a mixture of borax-carmine and Lyon's blue as described in a previous paper (King, 11). This stain gives particularly good results when it is used on freshly preserved material, as then all of the nuclei become dark red, the ectoderm and mesoderm appear dark blue, while the yolk cells take but a pale blue tint and, therefore, are easily distinguished from the other cells. This sharp definition of the tissues was of great assistance, particularly in the study of the sections of *Bufo*. All of the drawings given in the present paper were outlined with the aid of a camera lucida.

Bufo lentiginosus.

When the circular blastopore is closing in, the mesoderm, already differentiated from the other tissues, forms a continuous sheet of small, angular, slightly pigmented cells across the dorsal wall of the archenteron. In the middle and also in the anterior part of the embryo, the mesoderm is entirely separated from the ectoderm above as well as from the endoderm beneath it. In the region just in front of the blastopore, the mesoderm is also

distinct from the ectoderm, but it is united for some distance with the cells forming the dorsal wall of the archenteron. At this stage of development there is first noticed, in the middle of the embryo, a pronounced thickening of the mid-dorsal mesoderm (Fig. 1, *N*), which extends only over a few sections at first and is continuous with the lateral mesoderm on either side. When the blastopore is nearly closed, the thickened portion of the mesoderm is cut off from the lateral mesoderm to form the notochord, the line of separation coming in at about the points marked *XX* in Fig. 1. As the embryo elongates, the forward extension of the

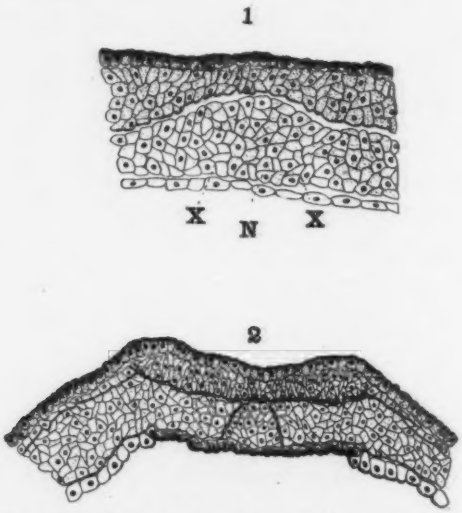
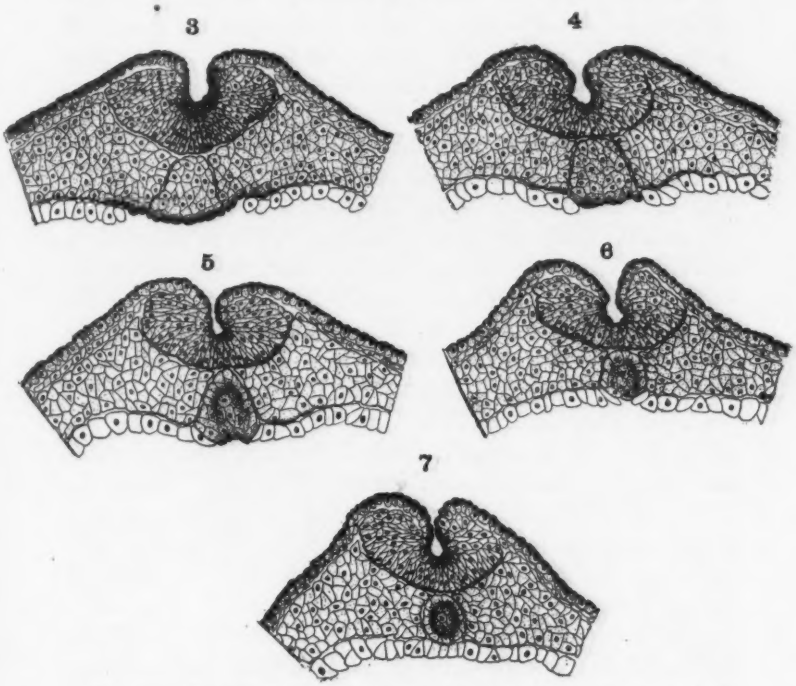


FIG. 1. Part of a medium sagittal section through an egg of *Bufo lentiginosus* in which the blastopore has begun to close. *N*, thickening of mid-dorsal mesoderm which is to be cut off at the points *XX* to form the notochord.

FIG. 2. Part of transverse section through the posterior region of an embryo in which the medullary plate has appeared.

notochord always takes place in this same way, *i. e.*, by the cutting off, laterally, of a portion of the mesodermal layer in the mid-dorsal region so that, from the beginning, the notochord is entirely separated from the ectoderm and also from the endoderm. These observations confirm the statement made in a previous paper (King, 12) that "the anterior part of the notochord is certainly mesodermal in origin."

Transverse sections through an embryo in which the medullary plate has just appeared show that, in the anterior region, the notochord is composed of a rounded mass of cells cut off entirely from the surrounding tissues, and appearing much as in Fig. 7. In the posterior region, there is, as yet, no trace of a notochord, and an unbroken layer of cells extends across the dorsal surface of the archenteron, as here the mesoderm is still



FIGS. 3–7. Serial sections from the posterior to the middle region of an embryo of *Bufo lentiginosus* in which the medullary folds are closing.

united with the endoderm as in the earlier stages. In a section made a short distance behind the middle of the embryo (Fig. 2), the notochord appears as a triangular shaped chord of cells, entirely distinct from the mesodermal layer on either side, but closely connected with the cells forming the mid-dorsal wall of the archenteron. In this part of the embryo, as well as more

posteriorly, the archenteron is surrounded on its ventral, lateral and lateral-dorsal surfaces by large, rounded, faintly staining yolk cells which contain very little, if any, pigment; the mid-dorsal wall, on the contrary, is formed of a single layer of much smaller, rectangular cells which are very heavily pigmented on the side bordering the archenteron. This layer of cells, which I shall call "the dorsal plate," is broadest in the posterior part of the embryo, where, in transverse sections, it appears as a nearly straight line of cells covering about two-thirds of the mid-dorsal surface of the archenteron. More anteriorly the dorsal plate gradually becomes narrower, until it finally disappears completely in the middle of the embryo. The archenteron in front of this region is entirely surrounded by large yolk cells.

The outer cells of the dorsal plate, instead of grading into the yolk cells as one might expect, are found to be directly continuous with the lower layer of mesoderm. There is, therefore, in this region an abrupt change from the small, deeply pigmented cells of the dorsal plate to the large yolk cells which form the lateral and ventral walls of the archenteron. At no stage in the development of the embryo have I ever found any transitional stages between these two different kinds of cells. The cells of the dorsal plate resemble, in all respects, the cells forming the outer surface of the embryo, being of the same size and shape and containing about the same amount of pigment. From the results which I obtained in my study of the gastrulation of the egg of this species (King, 12), it seems highly probable that the cells composing the dorsal plate were invaginated from the surface of the egg during the formation of the blastopore, and, consequently, they have had a very different origin from the cells forming the lateral and ventral walls of the archenteron which are all derived from the yolk portion of the egg.

When the medullary folds are closing, the mesoderm in the posterior region is still connected, for a short distance, with the cells forming the dorsal wall of the archenteron, and the notochord has not yet extended into this portion of the embryo. Fig. 3 shows a portion of the section through the region where the notochord has just been cut off from the mesoderm. This section corresponds in its position in the embryo with the position

of the section of the earlier embryo shown in Fig. 2. The notochord is triangular in shape and is closely connected with the layer of cells forming the mid-dorsal wall of the archenteron. The portion of the dorsal plate directly under the notochord is cut off on either side from the rest of the layer, and to it one can, perhaps, fitly apply the term "chorda-endoderm," since it is destined to become a part of the notochord. At this stage of development, the dorsal plate is much narrower in the posterior region of the embryo than it was before the medullary folds formed (Fig. 2), and it is again found to be directly connected with the lower layer of mesoderm and not with the yolk cells forming the lateral walls of the archenteron.

In Fig. 4, a portion of a section slightly anterior to that shown in Fig. 3, the chorda-endoderm is seen to be the only portion of the dorsal plate bordering the archenteron. The other cells of the dorsal plate have united with the mesoderm, and can only be distinguished from it on account of their position and the fact that they contain somewhat more pigment. The entire dorsal wall of the archenteron, excepting the part formed by the chorda-endoderm, is here composed of large, rounded yolk cells which are evidently growing up from both sides, and thus shutting off all of the cells of the dorsal plate from bordering the archenteric cavity. More anteriorly, as shown in Fig. 5, the yolk cells of the upper wall of the archenteron are still closer together in the middle lines. In this part of the embryo the cells of the chorda-endoderm no longer form a nearly straight line at the lower edge of the notochord, but they have become an integral part of it, and most of their pigment is collected in the form of a pronounced ring around the center of the notochord.

Near the middle of the embryo (Fig. 6), the yolk cells have almost met under the notochord, which is smaller and more rounded than it is in the posterior part of the embryo. A section more anteriorly still (Fig. 7) shows that the yolk cells from the two sides of the archenteron have come together in the middle line under the notochord. As a result, the dorsal wall of the archenteron is composed entirely of a single layer of large yolk cells, and the cylindrical notochord above it is cut off entirely

from the surrounding tissues. In the head region, the relation of the tissues is practically the same as that shown in Fig. 7.

When the medullary folds have closed, there is found in the posterior region of the embryo a much narrower dorsal plate than that shown in Fig. 3, as more of the cells have been covered over by the upward growth of the yolk cells from the sides of the archenteric cavity. Anteriorly the dorsal plate grows narrower very rapidly and some distance back of the middle of the embryo the yolk cells have already come to surround the entire archenteron. By the time that the optic bulbs have formed, there is no longer any dorsal plate in the mid-dorsal wall of the archenteron and the notochord has no connection with any of the surrounding tissues.

These results show that the anterior part of the notochord in the embryo of *Bufo lentiginosus* is entirely mesodermal in origin; in the posterior part of the embryo, the greater part of the notochord is also derived from the mesoderm, but there is added to it a single layer of chorda-endoderm from the mid-dorsal wall of the archenteron. Back of the middle region of the embryo, the yolk cells grow up from the lateral walls of the archenteron and unite under the notochord, the cells of the dorsal plate thus cut off from bordering the archenteron, either unite with the notochord or are incorporated into the splanchnic mesoderm.

Rana palustris.

In the frog, *Rana palustris*, the notochord is formed at about the same stage of development that it is in *Bufo*, namely, near the end of gastrulation when the blastopore is closing in. As in the embryo of *Bufo*, the notochord first appears in the middle region as a rounded chord of cells cut off from the mid-dorsal mesoderm, and it is separated entirely from the ectoderm and also from the endoderm beneath which forms the dorsal wall of the archenteron. At this stage in the development of the egg, the mesoderm in front of the region where the notochord has been cut off forms a solid layer of cells extending across the dorsal wall of the archenteron and entirely separated from it; the mesoderm back of the notochord also extends in an unbroken sheet across the mid-dorsal region, but in this part of the egg meso-

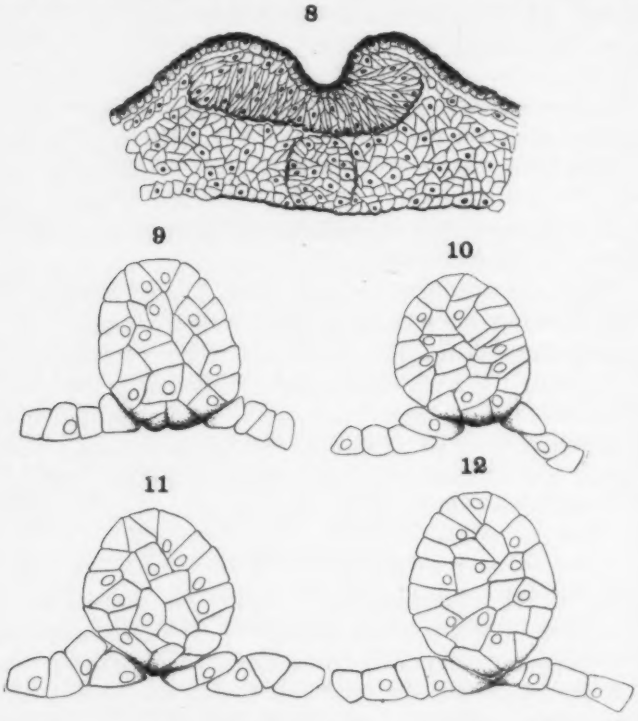
derm and endoderm are connected for a considerable distance on either side of the middle line.

In the posterior part of the embryo the cells forming the dorsal wall of the archenteron do not differ in size, shape, or in power of staining from the mesodermal cells above them, and at the sides of the archenteron they grade into the larger yolk cells forming the ventral and lateral walls. There is a comparatively narrow region in the mid-dorsal wall where the edges of the cells bordering the archenteric cavity are quite heavily pigmented; but the other cells of the dorsal wall contain about the same amount of pigment as do the mesoderm cells above them, and there is no definite dorsal plate of small, deeply pigmented, rectangular cells as in the toad embryo. I can find no evidence that any of the cells of the dorsal wall of the archenteron ever form a permanent union with the mesoderm.

When the medullary folds are beginning to form, the notochord has extended into the posterior region of the embryo and appears as in Fig. 8. It is a more rounded structure than is the notochord of the toad embryo at a corresponding stage of development (Fig. 3), yet it, too, is closely connected with the endodermal layer of cells forming the mid-dorsal wall of the archenteron. As shown in Fig. 8, the lateral mesoderm and the endoderm of the archenteric wall are connected for some distance on either side of the notochord. The cells of both of these tissues have the same general characteristics, and there is no sharp distinction between them as in the embryo of *Bufo*. As all of the cells in the dorsal part of the embryo have the same power of staining, it is not easy to follow the changes that take place, even with an abundance of material for study. Not until I had made camera drawings of a complete series of sections through the posterior region of an unusually favorable embryo was I able to tell with certainty how the notochord is formed. Four of these drawings (from the same embryo as Fig. 8) are reproduced in Figs. 9–12. For the sake of clearness only the dorsal wall of the archenteron and the notochord are shown. In al of the sections the mesoderm is entirely cut off from the notochord, and also from the endoderm beneath it.

A short distance in front of the region shown in Fig. 8, almost

all of the pigment in the mid-dorsal wall of the archenteron is found to be massed in the outer edges of a very few cells which are entirely cut off from the other cells of the archenteric wall and are attached to the lower surface of the notochord (Fig. 9). These few cells are undoubtedly comparable to the layer of chorda-endoderm found in the mid-dorsal wall of the archenteron



FIGS. 8–12. Serial sections from the posterior to the middle region of an embryo of *Rana palustris* in which the medullary folds are closing.

in the toad embryo, and, therefore, the same term may fitly be applied to them. More anteriorly (Fig. 10) there is a noticeable upward bend in the mid-dorsal wall of the archenteron, and it appears as if the notochord with the chorda-endoderm cells is either pulling in or being pushed in from bordering the archenteric cavity, while the cells of the dorsal wall of the archenteron

on either side of the notochord are coming together under the notochord. A few sections beyond (Fig. 11), the notochord is almost entirely cut off from the archenteron, as only one or two heavily pigmented cells lie between the two parts of the dorsal endoderm. In the middle region of the embryo (Fig. 12), the endodermal cells have united under the notochord and the notochord is a rounded chord of cells entirely separated from the surrounding tissues.

In *Rana palustris*, therefore, as well as in *Bufo lentiginosus*, the notochord is composed entirely of mesoderm in the anterior part of the embryo, and of mesoderm and chorda-endoderm in the posterior region. The early stages in the formation of the notochord are very similar in the two species; but in *Rana* there is no upward growth of yolk cells as in *Bufo* to form the permanent dorsal wall of the archenteron.

Most of the embryologists who have studied the early development of the Urodela agree with Jordan (10) who describes the formation of the notochord in the common newt as follows: "The cells of the median dorsal wall of the archenteron assume a somewhat columnar form and are gradually pushed up and pinched off until they are completely separated from the endoderm and come to lie above it in the mid-line." This view is held by Hertwig (7), Scott and Osborn (20), Field (5), Eycleshymer (4), Brachet (2), and Schwink (19).

Lwoff (13) is, perhaps, the most prominent of those who oppose this view. In his study of *Axolotl*, Lwoff finds that the mesoderm and the notochord are derived from cells invaginated from the surface of the egg at the blastopore rim, and he states: "Bei den Urodelen bildet sich die dorsale Wand des Darmes, ebenso wie bei Petromyzon, verhältnissmässig spät, nämlich nachdem die Chorda sich von den seitlichen Mesodermplatten gesondert hat. Die Entodermzellen wachsen von rechts und links einander entgegen, vereinigen sich unter der Chorda und bilden aufsolche Weise die dorsale Wand des Darmes." This description of the manner in which the permanent dorsal wall of the archenteron is formed in the *Axolotl* agrees remarkably well with the results of my investigations on *Bufo*. Lwoff's summary of the results of his study of the Anura based on an

investigation of the early development of *Rana*, is in part as follows: "Bei den Anuren liegen insofern anderen Verhältnisse vor, also hier die Zellen, welche die dorsale Wand des Darmes bilden, von Anfang an vorhanden sind ale eine Zellenreihe und zwar als eine untere Zellenreihe jener Anlage, aus welcher die Chorda entsteht." Lwoff and I are therefore in accord in believing that in *Rana* there is no upward growth of the yolk cells from the lateral walls of the archenteron to form the mid-dorsal wall.

There is great diversity of opinion concerning the manner of the formation of the notochord in the Anura; and, considering the careful work that has been done in this line, it seems highly probable that the process is not as uniform in this group as it is in the Urodela.

Goette (6), from his study of the development of *Bombinator igneus* concludes that in this species a central chord of mesoblast in the mid-dorsal region of the embryo separates from the two lateral sheets to form the notochord. This view is supported by the later investigations of Schultze (18), and Morgan (15) who worked on different species of *Rana*.

In a paper on the development of the middle germ layer in *Rana temporaria*, Hertwig (8) gives a number of figures of the posterior part of the embryo that bear a striking resemblance to those I have drawn of a similar region in the embryo of *Bufo lentiginosus*. Hertwig believes, however, that the entire notochord in the Anura as well as in the Urodela, is derived from a chorda-entoblast which at the sides of the archenteron pass into the endoderm cells forming the lateral walls. Field (5), from his investigations on *Rana temporaria* and on *Bufo vulgaris*, agrees with Hertwig regarding the manner of formation of the notochord, as do Robinson and Assheton (17) who worked on *Rana temporaria*. Balfour (1) also inclines to the same opinion, although he states that his evidence for so doing is not entirely conclusive.

As a result of his study of the early development of *Bombinator igneus*, Perenyi (16) advances still another theory regarding the formation of the notochord. He states that, when the blastopore closes in, "die vertikal nach innen vordringenden Zellen

der Deckzellen, welche zwischen beiden Teilen des Mesoderms liegen einander berühren und sich auf der dorsalen Seite von den äussersten Zellen abzuschnüren beginnen." In this way a rod of cells is cut off from the inner layer of ectoderm to become the notochord. I know of no other investigator whose results agree with those of Perenyi.

The results which Schwink (19) has obtained from his investigations on *Rana temporaria* and *Bufo vulgaris* are very similar indeed to those which I have recorded in the present paper for *Rana palustris* and *Bufo lentiginosus*. According to Schwink, the anterior portion of the notochord in *Rana temporaria* is entirely mesodermal in origin, while the posterior part has added to it a single layer of chorda-endoderm from the dorsal wall of the archenteron, the endoderm cells at the side of the notochord growing under and uniting in the mid-dorsal line. In *Bufo vulgaris* Schwink finds that the dorsal wall of the archenteron is composed of deeply pigmented cells which, at the sides of the archenteron, pass into the larger yolk cells, although he states that in some cases it appears " dass die hier liegenden Entoblastzellen aus dem bisherigen Verband scheiden um in den Mesoblast aufgenommen zu werden." Concerning the formation of the dorsal wall of the archenteron in the posterior part of the embryo Schwink states that, " hier von beiden Seiten Darmentoblastzellen gegen die Mittellinie streben und dass dadurch Zellen, die vorher den Darm dorsal auskleideten, mit zur Bildung der Chorda verbraucht werden." This agrees exactly with what I have found to occur in the posterior region of the embryo of *Bufo lentiginosus*.

Brauer's (3) studies on the development of the Gymnophiona show that, in the posterior region of the embryo, the upper wall of the archenteron is at first formed of cells which have been invaginated from the surface. These " animal cells " are sharply marked off from the yolk or " vegetative cells " which form the side walls of the archenteron. In the anterior part of the embryo, the archenteron is extended by its connection with the segmentation cavity which is bounded entirely by yolk cells. At an early stage of development, therefore, the dorsal wall of the archenteron in the anterior region of the embryo is composed of vegetative cells, while in the posterior region it is formed of cells invaginated

from the surface as I have found to be the case in the embryo of *Bufo lentiginosus*. At a later stage of development, vegetative cells grow up from the sides of the archenteron, and gradually cover up the invaginated animal cells which now form an unbroken sheet of mesoderm across the dorsal wall of the archenteron. A portion of this mesoderm in the mid-dorsal line is subsequently cut off from the lateral mesoderm to form the notochord.

In the posterior region of the embryo of *Bufo lentiginosus* a portion of the dorsal plate of cells which forms the mid-dorsal wall of the archenteron becomes cut off from the rest of the layer to be added to the notochord. If we attempt to trace the origin of this dorsal plate, we find that it is composed of cells invaginated from the surface of the egg before there was any division of the cells into ectoderm, mesoderm and endoderm. These invaginated cells form a part of the upper wall of the archenteron for a comparatively short period of development only, and those of the cells that are subsequently added to the splanchnic mesoderm soon lose their identity entirely, and cannot be distinguished in any way from the other cells of the mesoderm. The later history of the chorda-endoderm cells I have not followed.

As the endoderm cells that grow up from the sides of the archenteron and meet under the notochord are unquestionably derived from the yolk portion of the egg, the archenteron eventually becomes lined throughout its whole extent with yolk cells, and, therefore, the result is the same as if the archenteron was originally formed by a splitting between yolk cells as is believed to be the case by Robinson and Assheton (17), Houssay (9) and Moquin-Tandon (14).

According to Morgan, Wilson (21), Eycleshymer and others, there is an invagination of surface cells at the dorsal lip of the blastopore during the gastrulation of the frog's egg, and these invaginated cells come to form a part, if not all, of the dorsal wall of the archenteron in the posterior region of the embryo. In subsequent development, as the studies of Schwink and of myself show, these invaginated cells are not covered over by an upward growth of yolk cells from the lateral walls of the archenteron as is the case in the toad embryo. A few of these cells

are added to the notochord, the rest, as far as I have been able to determine, remain as part of the permanent dorsal wall of the archenteron. I have never seen a section of an embryo that would warrant my stating that some of these cells become added to the mesoderm, although in the posterior region of the embryo endoderm and mesoderm are connected for a much longer time than they are in the embryo of *Bufo*.

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ON THE COAGULATION OF THE BLOOD OF SOME ARTHROPODS AND ON THE INFLUENCE OF PRESSURE AND TRACTION ON THE PROTOPLASM OF THE BLOOD CELLS OF ARTHROPODS.[1]

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I. The Normal Coagulation of the Blood in Some Arthropods.

For this work the blood of *Limulus polyphemus*, *Homarus Americanus*, *Platyonychus ocellatus*, and some other arthropods, was used.

Coagulation of the blood takes place in a somewhat different way in *Limulus*, lobster and spider crab.

In *Limulus* the blood coagulates as a bulky mass which in the following 24 hours contracts considerably. A second coagulation of the fluid expressed from the clot may take place, to a slight extent, forming very loose gelatinous masses around the central clot, or more rarely, the whole serum may afterward coagulate.

In the lobster the first coagulum is usually relatively small, consisting of shreds; usually however, in the course of twenty minutes to an hour, the serum remaining after the first coagulation coagulates as a solid gelatinous mass which in the next twenty-four hours retracts but little. Very rarely no coagulation of the serum remains after the first coagulation takes place.

In the spider or lady-crab, the first coagulation is similar to that of the lobster blood. No second coagulation however takes place.

A combination of the following factors comes into play in producing the clotting of blood in arthropods.

[1] This communication is in most parts only an abstract of a fuller report which shall appear later. It is intended to continue these experiments.

1. *The Agglutination of Blood Corpuscles.*—This phenomenon may be observed uncomplicated by the other factors, *e. g.*, if we collect blood in distilled water, in solutions of gelatine or of adrenalin. Under these conditions the cytoplasm of the cells itself furnishes a sticky substance necessary for agglutination. Coagulation of the blood of arthropods is partially based on *the agglutination of the blood cells taking place spontaneously outside of the body.*

2. Just as the cells agglutinate, so the cell protoplasm having left the ruptured cell body agglutinates, and the remaining part of the cell frequently has the appearance of still intact but smaller cells. The protoplasm of cells which have been absolutely dissolved may also agglutinate and form gelatinous masses.

3. About six to ten minutes after the shedding of the blood the blood cells, which in the meantime have been mostly collected in strings, begin to move in contact with solid bodies, *e. g.*, the surface of the slide; the protoplasm of the cell becomes more and more drawn out, so that ultimately a fine network of flattened cells is formed in which for a certain time the anastomosing exoplasm can be distinguished from the granular endoplasm.

4. There also takes place the coagulation of a substance which previously was in solution. This coagulation is especially marked in the blood of the lobster, but it also takes place to a less degree in the blood of *Limulus*.

II. On Some of the Factors Determining the Second Coagulation of the Blood of the Lobster.

The conditions under which the coagulation of the substance first in solution in the blood of arthropods takes place can best be studied in the blood of the lobster. If, after the shedding of the blood, one quickly collects the first fibrin by shaking the vessel and then removing it with needles, the coagulation can be inhibited for longer or shorter periods, or even be entirely prevented. The fact that coagulation nevertheless usually takes place, although somewhat later, is partially to be explained by the fact that it is impossible to remove all shreds. These shreds mostly consist of agglutinated cells. If one examines the second gelatinous coagula formed at the bottom of the dish, even after

having apparently most carefully removed the shreds, one frequently sees under the microscope a small mass of blood corpuscles which had not been removed from the blood, in the center of a small gelatinous coagulum.

In many cases, however, a second coagulation after having removed most of the shreds does not take place even if some of these shreds have remained. In the cases in which it does take place it is also possible that the cell protoplasm, derived from the solution of so many blood cells, may be sufficient to cause coagulation even if the absolutely intact blood cells themselves have been entirely removed. It may be found that by adding water to the blood during the first coagulation and afterwards filtering the diluted blood, the second coagulation can be inhibited with quite a certainty for a longer period than otherwise, or may be even entirely prevented. Dilutions of the blood therefore produce an absolute diminution in the formation of fibrin for a certain period, not only a relative one in proportion to the remaining fluid.

In the experiments made such diluted serum was afterwards distributed in small dishes, 4 or 5 cc. into each dish; different substances were added to several of those dishes to determine the influence of these substances on coagulation.

It could be shown that the addition of about two or three pieces of shreds of fibrin derived from the first coagulation of the lobster blood had a very pronounced effect on the coagulation of the diluted serum. Without the addition of such fibrin coagulation either did not take place at all, or only after some hours or on the next day. The difference in the dishes with shreds of fibrin and those without the fibrin was very marked. The influence of these shreds derived from the first coagulation was a localized one, the clotting always beginning directly around the pieces of fibrin and from here advancing concentrically towards the periphery of the fluid.

The fibrin however produced by the first coagulation is not the only substance having such an effect; pieces of muscle of the lobster act about equally well. In these experiments the muscle was carefully washed so that all blood was removed. The fibrin of the blood of rabbits or rats, or muscle of frogs, was without

effect. The fibrin of these latter animals was used as the fibrin of the lobster, or in some instances was for two hours put into lobster serum previous to the experiment, so that substances present in the fibrin which might inhibit coagulation were removed. Lobster fibrin, treated in this way, was active; the fibrin of rats and rabbits was without effect. If, however, to the lobster serum previously treated with fibrin of rabbits, fibrin of lobster was added, the serum coagulated; therefore, no substance inhibiting coagulation had been extracted from the mammalian fibrin.

Small pieces of the pancreas-liver of the lobster entirely prevented, as was to be expected, coagulation.

After pieces of the first lobster fibrin had been put for ten minutes or longer into absolute alcohol, then washed and dried, they lost almost entirely their power to produce coagulation. A trace of the second gelatinous coagulum, however, was sometimes still formed around such pieces. Control experiments showed that alcohol passing into the serum was not the cause of this loss of efficiency.

Pieces of fibrin put into chloroform water over night and even for three days, may still be very efficient, though not quite as active as fresh fibrin. Put into chloroform water for five days they lost to a large extent their efficiency. The addition of 1 cc. of chloroform water to 4 or 5 cc. of serum, or of a few drops of pure chloroform, to the serum had no more influence on the coagulation than the addition of an equal amount of water.

Heating of the fibrin in the water-bath to 46 or 47° for 30 to 40 minutes, destroyed its efficiency to a great extent, though not completely. Heating up to 51–54° for 40 minutes, destroyed its efficiency entirely or almost so.

Heating of the serum to 46–50° during 30 minutes, usually prevented the spontaneous coagulation of the serum. If, however, one adds to such serum previously heated, fresh fibrin, the serum coagulates almost as rapidly as normal serum.

The addition of 1 cc. of glycerine to 4 cc. of serum did not have any specific inhibiting influence. Also the addition of 1 cc. of a weak solution of pure gelatine did not have any specific influence.

The addition of 1 cc. of water and 6–4 per cent. sodium chloride solution to the serum had in most cases but a slightly inhibiting effect.

In such solutions coagulation has occurred occasionally even more rapidly than with the pure serum; an accidental admixture of a larger number of blood cells to this liquid may perhaps have been the cause. All these experiments, as well as most of the following ones, were made with the addition of shreds of fibrin obtained from the first coagulation of the lobster blood.

The addition of 1 cc. of a 1 per cent. solution of potassium cyanide or of a 16 per cent. solution of Witte's peptone, or of a saturated solution of urea, had a decidedly inhibiting effect. The peptone solution prevented the coagulation entirely; the potassium cyanide and urea solutions frequently had only an inhibiting action for some time, after perhaps 16 hours (sometimes earlier) coagulation usually occurring. Generally only half an hour after the addition of these substances their effect became noticeable. The order in which these substances, according to their strength, acted on the serum, was the following: Potassium cyanide had the weakest and Witte's peptone the strongest action; urea stood between these substances.

If, however, the experiments were made in this way, that the pieces of the first fibrin were in the beginning put for some time into potassium cyanide, urea or peptone solutions, then after having been washed out, put into dishes containing the blood serum, the order of the loss of efficiency of the fibrin was reversed. Pieces having been kept for one half hour to two hours in peptone solution, were almost as effective as fresh fibrin, even pieces having been kept in peptone over night were frequently still very active, although in many cases they had lost a considerable part of their efficiency. It is of importance that the peptone be well washed out for some hours, otherwise the peptone, becoming extracted and dissolved in the serum, prevents its coagulation.

Pieces having been kept for one half to two hours in a solution of urea and having been afterwards well washed, have lost the greatest part of their efficiency, and after having been kept for twelve hours have become absolutely ineffective.

A 1 per cent. solution of potassium cyanide usually destroys the efficiency of the fibrin inside of half an hour. After having been kept for two hours in such a solution the fibrin becomes opaque.

One cubic centimeter of a 2 per cent. solution of potassium oxalate, or 5 drops of a 10 per cent. solution of the same solution added to 4 cc. of serum, entirely prevents the coagulation.

One half cubic centimeter of a 2 per cent. solution of potassium oxalate inhibits the coagulation markedly.

The addition of 5 drops, or 1 cc., or 2 cc. of a 2 per cent. calcium chloride solution had, in one series of experiments, a slightly accelerating influence on the coagulation in comparison with control experiments which were made with the addition of 1 cc. of a weak sodium chloride solution. Calcium is probably present in a sufficient quantity in the serum and the farther addition of it can no longer exert a pronounced influence.

A more extended series of experiments on the action of different salts on the coagulation is to be made later. Some experiments have, however, already been made on the action of ammonium chloride (the marked action of urea suggested these experiments). This salt, compared with the corresponding solutions of sodium chloride, has a specifically inhibiting action on the coagulation. The addition of 1 cc. of a 16 per cent. solution of sodium chloride frequently had a weak, though at times a somewhat stronger, inhibiting influence; 7 drops of a 16 per cent. ammonium chloride solution, or 1 cc. of a 4 per cent. solution of the same solution, had a much more pronounced inhibiting influence. One cubic centimeter of a 16 per cent. solution of acetamid had but a slightly inhibiting effect. The addition of a few drops of ammonium prevented coagulation entirely; this, however, was probably merely an alkali-action.

If the mixtures of blood serum with the above substances are kept for 24 hours in a warm room, putrefaction of the liquid usually takes place. This putrefaction does not prevent coagulation although it may inhibit it somewhat.

After the second, gelatinous, coagulation has taken place, it is possible to separate a second serum from this coagulum. This second serum, to which fresh shreds of fibrin have been added, no longer coagulates.

If, instead of the shreds of the first coagulation, the gelatinous coagulum of the second is added to the ordinary serum, efficiency of the latter fibrin is usually found not to be as pronounced as that of the first fibrin.

Foreign bodies, as such, added to the serum in place of fibrin or muscle, do not tend to accelerate coagulation. In no case could any marked influence be observed. Also platinum black was without any effect.

On the surface of the blood serum, kept in open dishes, usually a film is formed. This surface film in most cases radiates from the shreds of fibrin put into the serum. If we shake the serum for one half hour in dishes with uneven surfaces, macroscopically, visible membranes of fibrin are formed.

Extract of fibrin, which was previously kept in absolute alcohol, or of fresh fibrin under addition of chloroform water, have so far not shown an efficiency comparable to that obtained by the addition of fresh fibrin itself. One cubic centimeter of such an extract added to 4 cc. of serum, in most cases only caused a very insignificant acceleration of the coagulation in comparison to serum to which 1 cc. of a 6 per cent. sodium chloride solution had been added. Halliburton states that an extract of mammalian blood precipitated in alcohol and afterwards dried, produced coagulation in blood which had been collected in a magnesium sulphate soluion. I did not succeed in producing coagulation in this way ; such a result would be contradictory to the one obtained by myself with the fibrin of rabbits and rats.

We may explain all these facts by the supposition that in the shreds produced by the first coagulation there is present a ferment or pro-ferment producing coagulation, and further, that such a ferment or pro-ferment must be present inside of the blood cells, because the first clot consists mainly of blood corpuscles and parts of these cells. Further, we are certain of the decidedly localized action of this ferment, as directly around the shreds of fibrin the second gelatinous fibrin is deposited. This can be explained by the assumption that the ferment can only slowly diffuse into the fluid ; it must however nevertheless be able to diffuse through the gelatinous substance formed around the shreds, although as recent experiments seem to prove, even

soap mixed with gelatine is unable to diffuse through the gelatine.

The local apposition of the gelatinous mass around the fibrin shreds produces a certain resemblance between the process of clotting and that of crystallization when caused by a crystal in a solution. If we make use of the hypothesis of the action of a ferment, we have to suppose, according to the results obtained by heating the serum, that also in the serum a small quantity of this ferment or pro-ferment is present; further, that the ferment is partially destroyed by heating it up to 46–47° C. and that it is almost entirely destroyed by heating it up to 50–51° C. There is a quantitative relation present, the coagulation taking place the more rapidly the more fibrin is added to the serum. Although absolute alcohol is used to obtain mammalian fibrin ferment, and also, according to Halliburton, that of arthropods, in these experiments absolute alcohol destroyed efficiency of arthropod fibrin ferment in a very short time.

We saw that different kinds of cells and cell products of the same species of animals had the same accelerating effect on coagulation, that however the corresponding cells of other animals were without effect. The lack of specificity in the one case combined with the specificity with regard to the species of animals used, is similar to the specificity and lack of specificity respectively found in the precipitin reactions obtained after the injection of albuminous substances in different animals.

Delezenne found that contact with the muscle causes a coagulation of the blood of birds. If the contact with muscle is avoided, it is possible to keep the blood of birds liquid for a long time, although the white blood corpuscles are still present. Lobster blood, however, in contact with the blood corpuscles, does coagulate just as quickly as if in contact with muscle. These observations, notwithstanding the presence of some minor differences, seem to point out that in these phenomena we have to deal with facts of a general significance.

III. The Conditions Under Which the First Coagulation Can be Inhibited or Entirely Prevented.

The conditions under which the first coagulation can be prevented are different from those described above for the second

coagulation. The following experiments were mainly made with adult Limuli:

(*a*) If one collects blood at once into saturated solutions of different salts, surpassing in quantity considerably the quantity of blood received, coagulation can be to a great extent inhibited or prevented. Magnesium sulphate, sodium chloride, potassium chloride, sodium nitrate, aluminium, ammonium sulphate, potassium oxalate, sodium citrate, potassium and sodium tartrate, potassium sulphate and sodium sulphate, all have a decidedly inhibiting effect, and to a less degree sodium carbonate.

If the solutions are diluted with one half or an equal amount of water, the effect shows a corresponding decrease. Potassium salts are less effective than sodium salts; the potassium nitrate is almost without effect; the oxalates do not have any markedly stronger effect than the other saturated salts; it is therefore improbable that oxalates act in this case as precipitants of calcium, but rather as saturated salts. The blood corpuscles are in the main better preserved in these solutions. In the sodium and potassium sulphates they become roundish and send out processes after some time. In sodium nitrate and potassium and sodium chloride they retain on the whole an oval shape. Sabattani's hypothesis, that the inhibiting effect of salts is based on their action on calcium, preventing it from being ionized, becomes improbable, at least in this case. If the saturated solutions of the salts containing the blood are afterwards diluted with distilled water, many cell granules which had previously been preserved, now disappear. Many cells, however, seem to remain unchanged.

(*b*) Pyrrhol, resorcin and especially hydrochinon, have some inhibiting action on coagulation without preventing it entirely. Blood corpuscles remain more or less preserved in these solutions.

(*c*) Solutions of adrenalin chloride, 1–300, pilocarpine 1–300, and atropine 1–300, have a slightly inhibiting effect on coagulation, especially adrenalin. The cells here swell up and frequently arrange themselves through agglutination in epithelial-like order. Many cells, mainly at the margins where the adrenalin comes in contact with the blood, remain isolated or form only small clumps. The formation of a continuous coagulum however, at the margin does not take place frequently.

(*d*) A 3 per cent. solution of formalin injected into lobsters or spider-crabs has, if the blood is shed soon afterwards, a more or less inhibiting effect on the coagulation, especially if the blood is then also collected in a 3 per cent. formalin solution.

Under such conditions it can be observed that all blood corpuscles of the lobster blood have granules, and that cells corresponding to lymphocytes are normally not present in the blood of the lobster. Their presence has lately been maintained.[1]

(*e*) The first coagulation of the blood can be prevented by previously heating the animals; the heat necessary to produce this is different in different species. The blood of small Limuli heated for 30–40 minutes up to 50–54° C. does not usually coagulate after having been shed. Lobster or *Libinia*, heated to 45–48° C. for 30 minutes loses its coagulability. Correspondingly, lobsters and spider-crabs die at a lower temperature than small Limuli. The cells usually leave the body after the animal has been heated, in a round shape and later do not usually send out pseudopodia, or do so to a less degree only. The spreading out of blood corpuscles which usually takes place under normal conditions does not take place after previous heating of the animals, or if the animal has been injected with formalin.

(*f*) Blood collected in the liver-pancreas juice does not coagulate to the same extent as ordinary blood. A large number of the blood corpuscles are usually rapidly changed into granules and many of them soon become entirely dissolved through the action of the proteolytic ferment.

(*g*) By previous injections of *Limulus* blood into turtles I have not succeeded in obtaining either precipitins for *Limulus* blood or substances which inhibit the activity of its fibrin ferment.

(*h*) *Limulus* blood collected in gelatine solutions of different concentration did not coagulate as readily as the blood collected in water, gelatine having a decidedly inhibiting action. If the solution of gelatine was very weak care had to be taken to avoid the shaking of the blood, otherwise coagulation took place. The blood corpuscles in gelatine were swollen and frequently formed epithelial-like rows.

[1] A fuller description of the blood corpuscles of *Limulus* is given in a previous paper: *Journal of Medical Research*, Vol. II., January, 1902.

(*i*) Collected in glycerine or in chloroform the blood coagulates.

(*j*) The blood of the lobster collected in saturated urea solutions or in a 25 per cent. peptone solution, forms a gelatinous mass which does not contract in the usual way. Urea solutions have a strongly dissolving influence on the blood corpuscles. The shreds resulting from the first coagulation of the lobster blood put into urea solutions, become transparent and swell; in glycerine they also become transparent but do not swell; returned into water they regain their usual characters.

IV. On the Influence of Mechanical Agencies on the Protoplasm of the Blood Cells of Some Arthropods.

It has been observed by previous investigators that the blood cells may send out long processes which can adhere to solid particles. It is however possible to produce a much greater change. The protoplasm of a little clump of blood cells can be transformed into a system of threads of different sizes. It is best to use for such experiments cells which have been well preserved and are somewhat swollen; it is also necessary to use blood in which it is possible to exclude the presence of an extra-cellular mass of fibrin. Such cells are found, *e. g.*, in blood collected in gelatine solutions or in adrenalin or, also, in hydrochinon.

The necessary traction can be applied either by putting a second slide on the first containing the blood in solution and separating the two slides after they have been pressed together or by whipping with a needle the blood cells on the slide. The whole process can be followed under the microscope; one can see how cells arranged in a row become transformed into a system of threads which no longer indicates that it is derived from cells. Sometimes however we can see in the center of such threads the nuclei of the cells, adapting themselves to the thickness of the thread, or they may bulge out of the center of the fiber as little spheres. Under such circumstances one single cell can be changed into a very long thread. It can also be seen that frequently spindle-shaped cells arrange themselves into

a single row and may form one long continuous thread passing over a large part of the slide.

Sometimes very little effort is needed to achieve such a transformation. If we move the solution containing a few drops of blood in one direction the flow of the liquid may be sufficient to arrange the cells into rows and draw them out into continuous, long threads.

Such fibrils are, however, not only formed from the protoplasm of the entire cell but they also may be formed inside of cells. This can be observed, for instance, if we exert traction by a needle in a network of cells spread out on the slide, *e. g.*, about half an hour after the blood has been shed. If we merely exert pressure on masses of cells we may change them into a structureless gelatinous mass. By exerting afterwards traction on such a gelatinous mass, using two slides, we can change this mass into a system of fibrils of different diameters; such fibrils can again be rolled up into a gelatinous mass. The protoplasm has in this case the consistency of a dough, differing however in that it is somewhat more elastic.

If we apply these mechanical agencies to cells immediately after they have left the body, we find the protoplasm more brittle and the cells can under these conditions by pressure easily be separated into small granular particles.

A transformation into fibrils can also be produced from the protoplasm which had previously left the body of the ruptured cell, or from the protoplasm of exploded cells. The same agencies, traction and pressure, are also able to make the granules of the cells disappear. Cells which had been previously kept for a longer period in saturated salt solutions or in formalin, no longer yielded such a fibrillar mass.

The physical properties of this fibrin-like mass are very similar to the fibrin formed through the coagulation of the extra-cellular part of the blood. The cellular fibrin can be drawn out, and as it is somewhat elastic, may afterwards slowly retract. It is also sticky. The cells themselves have similar physical properties. If they are spread out, as is usually the case, one half to one hour after coagulation they are kept under great tension, and if liberated from the surface of the slide by a needle they retract.

They also are sticky. A cell, hanging on a fiber after coagulation of the blood has taken place, sticks to the surface of the slide; after having sent out processes, if as a result of outside motion a pseudopodium of the cell touches the surface of the glass.

Very similar fibrils can be produced if we subject the blood serum of *Limulus* or lobster to the same mechanical agencies. It usually however needs more exertion to obtain the same result from these colloidal solutions than from the cells.

V.

Certain analogies may be pointed out which exist between certain processes during coagulation of the blood and certain other phenomena.

(*a*) Inside of the body blood corpuscles have an oval shape and do not send out pseudopodia. After having left the body certain changes in the environment take place, the blood cells send out pseudopodia, and somewhat later they begin to spread out over the whole surface of the slide, forming one continuous network. We see a certain analogy between this process and the emigration of leucocytes under so-called inflammatory conditions.

The changes which lead to the spreading out of the blood corpuscles of arthropods cannot, under the given conditions, be determined by chemotropic influences acting from certain directions. The chemical conditions on the slide are the same on all sides; nevertheless, the cells move and spread out. Inflammatory conditions in the higher animals also mean a change of the normal environment brought by toxic or other causes. Thus it may be that the leucocytes of vertebrates do not migrate primarily under the influence of chemotactically acting substances, but under the influence of certain physico-chemical factors which bring about changes in the state of the protoplasm of these cells; direct chemical stimulants may be added secondarily.

(*b*) During regenerative processes of the epithelium movements of the cells take place independent of preceding cell multiplication. The epithelial cells under these conditions usually move in contact with solid bodies, parts of which, as I have previously shown, may be taken into the cell body itself. It can be observed under

these conditions that the protoplasm of the epithelium is in very close connection with the solid body, for instance the coagulum forming a scab, and if one raises the scab from the wound parts of the epithelium may remain in contact with it and are raised with it. It is not unlikely that similar changes in the consistency of the protoplasm of the epithelial cells take place during regeneration, as take place in the blood cells after they have left the body, and the adhesion may in both cases have to be explained by a change in the consistency of the protoplasm which makes the cell or part of it sticky.

(*c*) We have been able to show that under the influence of traction the protoplasm of the cells assumes a fibrillar structure and that these fibrils frequently are continuous through a number of cells. Similar phenomena can be observed in many other cells. Epithelial fibrils, for instance, are not limited to one cell and especially under the influence of traction exerted during the regenerative process, the fibrils passing through more than one cell are drawn in the direction of the traction exerted and indicate therefore the movements of the epithelium. In a similar way we find fibrils passing through many cells, for instance in the heart muscle, in the developing white fibrous tissue and neuroglia and probably also in the peripheral nerves and in ganglia cells. The same mechanical factor bringing about fibrillation which can be observed experimentally in the protoplasm of blood cells, may be of importance also in these cases. The main factor is of course the specific structure of the different cells determining the specific structure of these fibrils.

(*d*) The blood corpuscles which in the body under normal conditions do not change their oval form, produce outside the body an agglomeration of cells which may be compared to a tissue. Under certain conditions, *e. g.*, in gelatine or in adrenalin, or even in distilled water, many cells form through agglutination an epithelial-like tissue; under ordinary conditions however the cells soon form intercellular substances and the appearance of connective tissue is produced. The network of spread-out cells closely resembles the reticular tissue. The exoplasm of the spread-out cells under the influence of slight mechanical agitation, takes on a fibrillar character. If we make sections through a coagulum in

which the blood corpuscles have spread out, the appearance of connective tissue is presented. We see apparently intact cells surrounded by fibrils. These cells, however, only represent the endoplasmodic part of the original blood corpuscles, the exoplasmatic part having been transformed into fibrils. Many of these fibrils stain very well by Mallory's connective tissue stain.

In a similar way, the fibers of fibrous tissue and of neuroglia in mammalians seem to be produced from the exoplasm of the cells. In coagulating blood, however, whole cells may be changed into fibers. Here protoplasm having left the cell body and being now entirely intercellular may also form fibrils. Such an occurrence can easily be observed under the microscope in the blood, these changes taking place in a short time. It would be more difficult to prove such an occurrence in the development of connective tissue. The morphological similarity (not identity) between the processes in the blood cells and connective-tissue cells being apparently so great, it ought to be considered, whether an intercellular origin of fibers from protoplasm, secondarily disconnected from cells may not also take place in connective tissue.

We can observe that the granules of the blood cells frequently spread out and assume in the end an intercellular situation, and that they not infrequently become included in fibrils. Similar observations have been made in the developing cartilage for instance.

As we have seen, the formation of intercellular substances in the coagulating blood can be prevented if we collect the blood cells in certain solutions, which inhibit that further changes in the cells take place without preventing the possibility of such changes, if the solution is changed afterwards. We may therefore say that one of the conditions under which the formation of an intercellular substance takes place is the presence of a certain fluid which surrounds the blood cells which have left the body. The serum has therefore a more or less cytolytic power and the formation of "connective tissue" is based on a partial destruction or dissolution (cytolysis) of the blood cells by which protoplasm leaving the cells becomes changed into intercellular fibrils.

Summary.

1. The following factors play a part in the coagulation of the blood of arthropods :

(*a*) Agglutination of blood cells.

(*b*) The formation of a gelatinous mass, and secondarily the formation of fibrils from protoplasm exuded from cells and from protoplasm of cells entirely dissolved.

(*c*) The spreading out of the blood cells during the next few hours after shedding of the blood.

(*d*) The coagulation of a fibrinogenous substance.

2. The agglomeration of the blood cells leads to the formation of tissue-like structures. The formation of the fibrillar intercellular substance corresponds to the formation of fibrillar connective tissue. By changing the cytolytic medium in which the cells are suspended, the formation of an intercellular fibrillar substance can be prevented. By agglutination of cells which remain well preserved, an epithelial arrangement of the blood cells can be brought about.

3. Inside of the normal body the blood cells do not change their form ; this however takes place outside of the body, without the presence of certain specifically directing chemotropic stimuli. The cell protoplasm moves in contact with the solid surface to which it sticks. In a similar way the leucocytes react during an inflammatory process and a certain analogy also exists between this process and the movement of the epithelium during regeneration.

4. Coagulation of the blood can be prevented or inhibited by receiving it in different solutions, such solutions usually having also a preservative influence upon the cells. Oxalates act in a similar way to other salt solutions, that is if they are very concentrated. This points to the fact that in all probability the precipitation of calcium is, under these conditions, not the essential factor. Sodium and potassium sulphate have, in a similar way as sodium nitrate and sodium and potassium chloride, an inhibiting influence upon coagulation. The blood cells themselves, however, are influenced in a different way by the two sulphates and the other salts.

5. The coagulation of the blood is inhibited by receiving the blood in gelatine solutions ; collecting it in oil is without influence.

6. The second coagulation of the lobster blood is inhibited by means different from those acting on the first coagulation. The

facts observed during the second coagulation can be explained through the hypothesis of the action of a fibrin ferment present in the blood cells and in the muscle of the lobster, which however is not present in the blood cells or in the muscle of certain vertebrates. This points to a multiplicity of fibrin ferments in different species of animals and points to the identity of the ferment in different tissues of the same species of animals. This specificity in the one case and the want of it in the other case corresponds to a similar specificity and want of specificity as has been found to exist with the precipitins obtained through injection of certain body fluids or albuminous substances into different species of animals.

7. Potassium cyanide, urea and solutions of peptone inhibit the second coagulation. If these substances are add to the serum their inhibiting influence is in a reverse proportion to thire strength if they act directly upon the fibrin which contains the ferment. Potassium cyanide has the strongest, peptone the weakest action upon the fibrin, and conversely, peptone has the strongest and potassium cyanide the weakest action on being mixed with serum.

8. The necessity of the presence of calcium for the second coagulation can be easily proved. Ammonium chloride has a stronger inhibiting action on the second coagulation than sodium chloride.

9. Mechanical agencies, namely, pressure and traction, change the cell protoplasm into a system of fibers which cannot be distinguished in its physical properties and appearances from the extra-cellular fibers. The cell granules disappear under the influence of traction and pressure, just as the granules disappear spontaneously in the cells during coagulation of the blood. By traction and pressure exerted upon the blood serum free from cells, similar fibrillar structures can be produced as in the protoplasm of the cell. The fibrillar structure of the protoplasm seems therefore to be a secondary condition, produced by mechanical influence upon albuminous substances which may be common to the protoplasm of the cell and to solutions of certain albuminous substances.

10. By pressure and traction the cell protoplasm can be changed into fibers whose arrangement is determined by the

direction in which the mechanical forces act; mechanical factors may also determine the direction of the fibrils in connective tissue and bone.

11. The significance of the blood corpuscles for the coagulation of the blood consists therefore in the following: (*i*) The cells themselves are transformed into substances similar to fibrin, (*ii*) They accelerate a coagulation taking place in the surrounding fluid; some facts point to the possibility that the coagulating substance contained in the serum has its origin in the blood cells.

12. Towards foreign substances introduced into the body of the animal, the blood corpuscles of *Limulus* do not behave as actively as the blood corpuscles of mammals. An active penetration into these foreign substances cannot be observed in *Limulus*, the cells only take part in the formation of the coagulum around the foreign substance.

PHOTOTAXIS IN VOLVOX.

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The reactions of *Volvox* to light have been studied in some detail by Oltmanns[1] who came to the conclusion that the phototactic movements of this organism are determined through the effort to reach a region of a certain optimum intensity of illumination. The theory at which Sachs and Strasburger had arrived from their studies of the phototactic reactions of plants, and which Loeb had applied to the phototaxis of animals, namely, that the direction of the movement is determined by the direction of the rays of light is not accepted by Oltmanns, but the experiments upon which this writer bases his confutation of this theory do not, I believe, bear out his interpretation. Oltmanns studied the reactions of *Volvox* to light of varying intensity by keeping specimens in a vessel so illuminated that the intensity of light gradually increased from one end to the other. This was accomplished by covering the vessel with a box the top and ends of which were made of wood while the sides were formed of narrow, wedge-shaped, hollow glass prisms filled with a mixture of India-ink and gelatine. The small ends of the prisms allowed most of the light to pass through, while the thick ends absorbed most of the rays. The light entering the enclosed vessel was obviously greatest opposite the thin ends of the prisms and gradually diminished toward the opposite end. The *Volvox* were found to assemble in different places in the vessel according to the intensity of light which fell upon the lateral prisms. With a comparatively dim outside light they would gather in the brightest end of the vessel; in light of very strong intensity they would seek the darkest end; while with moderate illumination they would take up some intermediate position. These results were held by Oltmanns to indicate that the *Volvox* seek the places of optimum intensity of illumination and remain there,

[1] Flora, 1892, p. 183.

much as higher organisms collect in situations where it is neither too warm nor too cold. In the experiments of Oltmanns we certainly cannot assume that the factor of the direction of the rays has been excluded. Owing to the scattering of the rays entering perpendicularly through the sides the light end of the vessel, in such an apparatus as Oltmanns employed, must act practically as an independent source of light. If, therefore, *Volvox* is positively phototactic in weak light and negatively so in strong, we may readily understand why collections are formed in regions of a certain intensity of illumination in accordance with the theory that the direction of movements is determined by the direction of the rays.

As an opportunity presented itself this last fall of procuring *Volvox* easily and in large numbers I endeavored to work out some points in the phototaxis of this organism a little more in detail, and, if orientation to the direction of the rays should be found to occur, as seemed probable from the statement of previous observers, to ascertain the method by which the orienting response is brought about. It is easy to determine that *Volvox* orients itself, and that very accurately, to the direction of the rays of light. If specimens of *Volvox* are taken into a dark room and exposed to the light from an arc lamp they travel towards the light in almost a straight course, swerving remarkably little to the one side or the other. They will often travel a foot without deviating as much as a quarter of an inch from a perfectly straight course. If the position of the light is changed during their progress they soon re-orient themselves and travel straight onwards as before. If the light is placed at the other end of the vessel they turn about and come back to where they started. The shape of the *Volvox* is not quite spherical but slightly elongated, forming a prolate spheroid, and when swimming through the water the organism rotates on its long axis. As is well known the anterior end of *Volvox* may be distinguished by the fact that it is usually free from daughter colonies, and Ryder has pointed out that the red ocelli of the zoöids are much larger at this end than elsewhere and diminish gradually in size towards the posterior end of the body. While swimming towards the light the largest ocelli are always directed towards the region of greatest illumination.

That *Volvox* is negatively phototactic in strong light may be determined by exposing it to direct sunlight, or to a beam from a projection lantern after eliminating the heat rays by means of an alum cell. In negative phototaxis the body is also definitely oriented, but with the anterior end away from the light, and the organism swims away in a very nearly straight course. When it reaches a place where the light becomes less than the optimum it stops and remains comparatively quiet, only moving about slowly at intervals in an irregular manner. In very weak light *Volvox* exhibits no pronounced phototactic movements, but either lies quiet or rolls about sluggisly in various directions. With stronger illumination it becomes more active and swims straight towards the source of light, while in light of high intensity the direction of the response is reversed. We may readily understand, therefore, why in the experiments of Oltmanns the *Volvox* formed groups in regions of a certain intensity of illumination. Collections would be formed if the *Volvox* moved about irregularly without regard to the direction of the rays and came to rest when they reached a region of a particular intensity of light; but it is clear that this is not the method pursued. There are few organisms in which the orientation to the direction of the rays is more precise, or which travel to or from the light in more nearly a straight line.

How is the orientation of *Volvox* effected? It is practically impossible to determine this by studying the movements of the flagella of the individual cells, as any one who has attempted to observe these movements will easily realize. We are safe in saying that when *Volvox* changes its direction it is because the flagella on the two sides of the organism beat unequally. Can we explain the orientation, then, as a result of the fact that the differences of intensity of light on the two sides of the body cause the flagella to beat with unequal vigor so that the organism is swung around into a position of equal bilateral stimulation? It is in this way that Holt and Lee[1] have attempted to explain the orientation of *Volvox*, but there are certain difficulties in the way of such an interpretation. Let us consider a *Volvox* in a region of suboptimal stimulation and lying obliquely to the rays

[1] *American Journal of Physiology*, Vol. IV.

of light. If it orients itself to the light the backward stroke of the flagella, *i. e.* the stroke that is effective in propelling the body forward must be more effective on the shaded side than on the brighter side. This may conceivably occur in the following ways, which, however, amount practically to the same thing: The diminished intensity of light on the shaded side of the body may act as a stimulus to the backward phase, or decrease the efficiency of the forward phase of the stroke of the flagella; or the light on the brighter side of the body may inhibit the backward phase, or increase the forward phase of the stroke of the flagella. In any case, if the organism is passing into regions of ever-increasing intensity of light, we should expect its rate of speed would be lowered. If the orientation is effected by a shading of the side away from the light it would follow that in a region in which the shading were less the speed of the travelling body would be diminished. If the parts of the body which are most shaded are the parts where the effective beat of the flagella is the strongest, then, as the organism passes to a point where the illumination on both sides of its body is increased, its rate of transit would be diminished. If we suppose that the forward stroke is most stimulated, or the backward stroke most inhibited on the brightest side of the body we should expect that with more illumination the more inhibition there would be, or the more the backward phase of the stroke would be increased, and the rate of locomotion would likewise be reduced. If we imagine a machine in the form of a *Volvox* colony and provided on all sides with small movable paddles so adjusted that when they came into regions of diminished light as the machine rolled through the water their effective beat would be increased, it is clear that such a machine might orient itself to the direction of the rays and travel towards the source of illumination, but its rate of locomotion would be diminished the brighter the light into which it passed. We may conceive the light to increase or decrease the backward or forward stroke of the paddles in any way we please and we cannot explain how such a machine can orient itself and go towards the light and at the same time move through the water more rapidly as it comes into regions of greater illumination.

Does *Volvox* react to light as the theory above mentioned

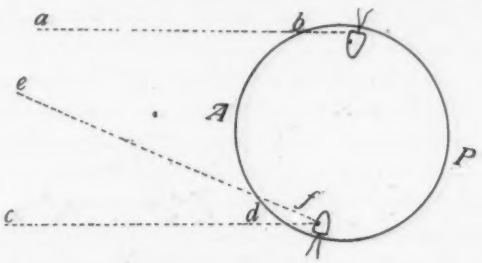
would lead us to expect? Oltmanns' observation on the relation of the rate of movement of *Volvox* to the intensity of illumination, while they apparently do not conform to this theory, were not made sufficiently in detail to form a crucial test of its validity. In order to obtain evidence which would be somewhat more conclusive I placed specimens of *Volvox* in a narrow glass trough through one end of which light from an arc lamp was passed after having filtered through an alum cell. The trough was placed over a paper ruled off in spaces a centimeter in width. The specimens were placed at such a distance as was found by previous trials was about the point where they would begin to orient themselves and travel towards the light. The number of seconds required by a specimen to travel across successive intervals in its passage towards the light was noted. The experiment was repeated many times, both by using different specimens and by using the same one over again. It was found that, as the *Volvox* travelled towards the light, their movement was at first slow, their orientation not precise, and their course crooked. Gradually their path became straighter, the orientation to the light rays more exact and their speed more rapid. After travelling over a few spaces, however, their speed became remarkably uniform until the end of the trough was reached where they would remain. If the light is so intense that one end of the trough is above the optimum intensity of illumination the speed of the *Volvox* is decreased as it approaches this optimum where it finally stops. In going away from very intense light *Volvox* moves at a nearly uniform rate until within a few centimeters of the optimum when the speed begins to diminish. There is thus a lessening of speed as the optimum is approached from either direction. The distance over which there is either a marked increase or decrease of speed is considerably less, however, than the space over which the speed is nearly uniform.

When we attempt to explain the foregoing facts on the theory that orientation is effected through the differences of the intensity of light on the two sides of the organism we inevitably get into difficulties. If the *Volvox* acts as a lens concentrating the rays on the side farthest from the light so that that side is more intensely illuminated the behavior of the organism would meet the

requirements of the theory. Orientation, however, occurs, apparently equally well, in those individuals which contain so many daughter colonies that a large proportion of the light is intercepted in passing through the organism; this explanation must, therefore, be dismissed even if it be otherwise valid. How then is the orientation of *Volvox* brought about? This problem is one rather more difficult to solve than it might seem. There is a suggestive similarity between the phototaxis of *Volvox* and the reactions of this organism to the electric current. Carlgren has found that *Volvox* orients itself very precisely to the constant current and swims in very nearly a straight path to the kathode. After a prolonged action of the current a more or less pronounced tendency to go towards the anode asserts itself, but the latter form of electrotaxis is much less precise and characteristic. It seems not altogether improbable that light in passing through a nearly transparent organism like *Volvox* exercises a directive effect upon its movements in a similar way, whatever it may be, to that produced by the current of electricity. The direction of the rays may be the important factor in orientation irrespective of differences of intensity of light upon different parts of the organism as has been maintained by Sachs for the phototropic movements of plants. I am not ready to adopt the theory of Sachs, but I feel that it is a view that is not entirely out of court.

There is one feature of the structure of *Volvox* which may be of some significance in relation to the problem of orientation. As Overton has pointed out, the red eye spots of the cells are so placed that they all face the anterior end of the colony. When this end is directed toward the light the spots are in a position to receive more than the usual stimulus. Orientation of the colony would be produced if each cell were to react in such a manner as to cause the eye spot to face the light. We have little direct evidence, aside from a single experiment of Englemann on *Euglena*, that the so-called eye spots of the Flagellata are photorecipient organs, but there are certain facts regarding the occurrence of these spots as well as their arrangement in *Volvox* which render it probable that such is their function. We may conceive that each cell of the colony tends to orient itself at a different angle to the rays of light, the cells of the anterior end where the largest eye spots occur placing themselves with their long axes

parallel with the rays, and the other cells at various angles depending upon their position in the colony. To account for the orientation of the colony we are thrown back upon the problem of the mechanism of the process whereby each individual cell places itself so that its eye spot faces the source of light. The behavior of the cells of the colony, according to this interpretation would fall into Prof. Mark Baldwin's somewhat extensive category of imitative activity, in that each cell reacts so as to secure more of the stimulus affecting a specialized portion of its structure. How and why the cells so react we still have to explain, and various theories of orientation may be applied to the individual cells. The orientation of the colony may be accounted



for rather more simply, however, if we suppose that the eye spots are most sensitive to light striking them at a certain angle such as is indicated in the diagram by the lines *ab* and *ef*. If rays of light enter the colony in the direction of the lines *ab* and *ef* somewhat obliquely to the long axis, *AP*, the flagella of the cell represented on the upper side of the diagram would beat more vigorously and accelerate the motion of that side of the organism. The opposite cell being struck by rays in the direction *cd* would be less stimulated, and, as the flagella would beat less strongly than those on the other side of the colony, the organism would swing about until its long axis is brought parallel with the rays when, being equally stimulated on both sides, it would move in a straight course towards the light. We do not have to suppose that each cell makes a special effort to orient itself at a particular angle to the rays, but that it is so organized that the effective beat of its flagella is most accelerated by light striking the cell at a certain angle. If the cells were most stimulated by light

falling upon them at such an angle as would result if the rays diverged from a spot in front of the colony and in line with its long axis the conditions for orientation would be fulfilled. Since the eye spots in all the cells face the anterior end of the colony this supposition appears very probable. The foregoing explanation of the orientation of *Volvox* may or may not be the true one, but it enables us to see a significance in the peculiar arrangement of the eye spots in this form and is consistent with the results of the experiments we have described.

How are we to explain the fact that *Volvox* becomes negatively phototactic in light of strong intensity? We certainly cannot explain the reversal on the supposition that it is due to a difference of emphasis in the phases of the stroke of the flagella. In positive phototaxis the backward phase of the stroke of the flagella is the stronger, and if we suppose that with increase of stimulation the reverse phase comes to predominate the organism would go backwards instead of forwards. As it is the anterior end of the organism that is directed away from the light in negative phototaxis it is obvious that the same phase of the stroke of the flagella predominates is both cases. The theory of Holt and Lee applies here very well; if the effective beat of the flagella is greatest on the more illuminated side the organism would naturally turn about so as to point away from the light. This may or may not be the true explanation of the negative reaction; I see at present no way either of proving or of disproving it. The probability of the supposition is somewhat weakened, however, by the fact that it cannot also be applied to positive phototaxis. It seems probable that light accelerates the action of the flagella not only by stimulating the eye spots but by acting on other parts of the cells, and we might suppose that, as the light is increased, a point would be reached where the stimulus to the other parts of the cell would outweigh the effect upon the eye spots, and the flagella on the side of the organism nearest the light, beating more vigorously than the others, would thereby bring about the negative orientation. We can thus give at least a formal explanation of the phenomenon. There are many instances in which an increase in the intensity of a stimulus causes a reversal of the usual reaction, but the reason for this in any case is still an unsolved problem.

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